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Pogonomyrmex anzensis Cole: Does an Unusual Harvester Ant Species Have an Unusual Venom?

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Abstract.—*Pogonomyrmex anzensis* was a lost "mystery" ant not seen or collected for 45 years after its original single collection, despite intense search by some of the finest myrmecologists of the time. Its rediscovery by a team in 1997 revealed the species nested in hard rocky hillside slopes that are exceptionally sun-baked, hot, and dry. Since these ants live under unusually extreme conditions compared to other members of the genus, we wondered if their unusual biological circumstances also translated into unusual venom. Compared to the venoms of most other species of *Pogonomyrmex*, the venom of *P. anzensis* is exceptionally lethal to mammals, but the amount of venom produced is low. The defensive behavior of *P. anzensis* reflects these venom properties: worker ants are unaggressive compared to other *Pogonomyrmex* spp. and their stings induce little pain or reaction in humans. Overall, *P. anzensis* is an atypical harvester ant species both in its habitat and behavior and in its reduction of venom production. The reduced venom production is likely a response to the combination of harsh conditions and an environment essentially free of vertebrate predators.

Key words.—*Pogonomyrmex*, *anzensis*, venom, lethality

Harvester ants in the genus *Pogonomyrmex* are among the most conspicuous arthropods within their habitats (Cole 1968; Taber 1998) and are famous for their exceedingly painful and long lasting stings (Schmidt and Blum 1978). Their venoms are among the most toxic of arthropod venoms, having lethalities many times greater than honey bees, most other stinging wasps and ants, and spiders and scorpions (Schmidt 1990). Harvester ant venom is used primarily for defense against large predators, and, with the exception of horned lizards in the genus *Phrynosoma*, worker *Pogonomyrmex* spp. have no major vertebrate predators (Schmidt and Schmidt 1989). This apparent freedom from vertebrate predation leads one to wonder if predatory pressure by vertebrates on ancestral *Pogonomyrmex* species was responsible for the incredible painfulness and lethality of harvester ant venoms and for maintaining that activity.

Pogonomyrmex anzensis lives in small, sporadic colonies in harsh desert rocky slopes around Anza Borrego in California, USA (Snelling et al. 2009). The ants are exceedingly timid, do not sting readily, are apparently allopatric to *Phrynosoma*. Thus, *P. anzensis* represents an interesting example of an unusual harvester ant species living in an extreme habitat free from even horned lizard predators. The goal of the research reported here was to determine if these conditions led to a loss of defensiveness and venom activity in *P. anzensis*.

MATERIALS AND METHODS

Pogonomyrmex anzensis workers were collected from two locations in Split Mountain, Anza-Borrego Desert State Park, San

Diego County, California: 2.7 km. S of jct. Split Mtn. Rd. and Fish Creek Rd., 33.02°N 116.10°W, 152 m, 2 April 1997, and at Split Mountain, 33.01°N 116.10 °W, 152 m, 26 April 1998; *P. wheeleri* Olsen were collected 18 km E. of jct. Mex. Hwys. 40 and 15 on Mex. 40, Mazatlan, Sinaloa, Mexico, 7 July, 1983; *Apis mellifera* L. were collected as foragers entering and leaving a feral colony near Cañas, Guanacaste, Costa Rica, 5 February 1987. Live ants were frozen and maintained at −26°C until dissected; the bees were treated similarly except the frozen conditions were −10°C. Pure venom was obtained from the frozen ants by the method of Schmidt (1995). In brief, a sting apparatus from a frozen ant was removed to a spot of distilled water, the venom reservoir (minus filamentous glands) was pinched off and removed from the rest of the sting apparatus, rinsed with distilled water, and placed in clean distilled water. Collected reservoirs were pooled in a single water drop, after which the venom was squeezed from each torn reservoir with pairs of forceps and the empty chitinous reservoirs were combined for weighing. The pure venom was lyophilized and stored at −26 °C until used.

Swiss white mice were used for lethality analyses and were provided food and water ad lib throughout the experiments. Venom was dissolved in 0.15 M saline and volumes of 0.6% of the mouse body weight were injected intravenously into groups of 6 (ants) or 8 (bees) mice. LD_{50} values (24 hr) were calculated according to the method of Reed and Muensch (1938) with 95% confidence intervals (CI) determined by the method of Pizzi (1950). The total lethal activity of the venom from single ants was expressed as the lethal capacity calculated by dividing the weight of venom per individual ant by the LD_{50} (Schmidt 1986). LC is expressed in terms of weight of mouse that would receive a median lethal dose of venom from the sting of one average ant.

RESULTS

Workers ants of *P. anzensis* are retiring and timid compared to other species in the genus. They make little effort to defend the nest when disturbed, and mainly run around erratically and excitedly before retreating. They also are hesitant to sting; in fact, so hesitant that the only stings (n = 3) received were those experienced by one of us (GCS) when individuals were pressed against the skin. The subsequent pain and reaction was milder and less severe than that experienced when stung by most other species of *Pogonomyrmex*. As seen under a dissecting microscope, venom reservoirs of *P. anzensis* often appeared collapsed or partially collapsed and only half filled or less with venom. Of 38 reservoirs scored, seven appeared empty, 11 one quarter full, 19 one half full and only one mostly full. The low filling of venom in the reservoirs corresponded to the low amount of dried venom per reservoir (Table 1). Another indication of low venom production and quantity in the species is the ratio of weight tissue in empty reservoirs to the venom within the reservoirs. The amount of venom per reservoir in *P. anzensis* was about 10% as much as for the congeneric *P. wheeleri*, whereas the ratio of empty reservoir tissue was roughly 10 times as much (Table 1). Virtually all other species of *Pogonomyrmex* exhibit venom to empty reservoir ratios similar to those of *P. wheeleri* (personal observations, JOS). Africanized ("killer") honey bees were chosen as a comparison outgroup. Their venom to empty reservoir ratio is similar to that of *P. wheeleri*.

The lethality of the venom of *P. anzensis* to the mouse vertebrate model is shown in Table 2. The venom itself is strongly lethal, exhibiting an LD_{50} of 0.20 mg/kg, three times more lethal than the venom of *P. wheeleri* or many other *Pogonomyrmex* species (unpublished data, JOS), and 10 times more lethal than that of the honey bee. A more realistic measure of venom effective-

Table 1. Quantity of venom in *Pogonomyrmex anzensis* and reference stinging Aculeata.

Taxon (location)	Material	n	Weight/insect (μg)	Empties/Venom
Pogonomyrmex anzensis	Venom	31	4.08	
(Split Mt Rd & Fish Cr.)	Empty reservoirs*	31	1.61	.395
(Split Mountain)	Venom	100	4.75	
	Empty reservoirs	89	1.24	.261
Pogonomyrmex wheeleri	Venom	41	46.2	
	Empty reservoirs	601	1.81	.039
A. mellifera (Africanized)	Venom	51	156	
	Empty reservoirs	51	13.7	.088

* Empty reservoirs consist of reservoir tissue with traces of residual venom

ness is lethal capacity, a measure of the killing power in terms of grams of animal that would receive a LD_{50} dose of venom if all of the venom in one individual were delivered in a sting. By this measure, a *P. anzensis* sting is less than one third as potent as one from *P. wheeleri* and less than half that of a honey bee.

DISCUSSION

For a harvester ant, the sting of *P. anzensis* is exceptionally mild to humans, with stings resulting in little more than mild pain and a small reddened area. The exceeding lethality of the venom itself indicates that the species has retained the ancestral venom activity observed throughout the genus. We do not have a species level phylogeny including *P. anzensis* and, therefore, cannot compare its venom activity to that of sibling species. In contrast to the extreme lethality of the venom, *P. anzensis* workers produce very little venom. Evidence for this is two-fold: the venom amount is small; and the reservoir that stores the venom is large and has the capacity to contain much more venom. A consequence of the combined lethality and low quantity of venom produced is a relatively low venom lethal capacity. These findings concur with field observations that the ants do not strongly defend themselves or their colonies and that their stings are not particularly effective as a potential deterrent to vertebrate predators.

Selection pressure can act on organisms living in harsh environments such as *P. anzensis* by either changing the nature of the venom itself, or by altering the control of venom production. We suggest that the evolutionarily more rapid and efficient

Table 2. Lethality and lethal capacities to mice of *Pogonomyrmex anzensis* venom and venoms of reference stinging Aculeata.

Taxon (location)	LD_{50} (mg/kg) (95% CI)	μg Venom	Lethal capacity insect (g mouse/sting)
Pogonomyrmex anzensis			
(Split Mt Rd & Fish Cr.)	.22	4.08	18.5
(Split Mountain)	.18	4.75	26.4
*Pogonomyrmex wheeleri** (Mazatlan, Mexico)	.60 (.38–.96)	46.2	77.0
A. mellifera (Africanized)** (Cañas, Costa Rica)	2.8 (2.0–4.1)	156	55

* Data from Schmidt and Schmidt (1985)
** Data from Schmidt (1995)

means of adapting to a harsh, essentially predator-free environment is to restrict investment of valuable energy and resources in venom production by limiting venom synthesis – something apparently occurring in *P. anzensis*.

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